ENGINEERED CHO CELL LINE WITH COXSACKIE AND ADENOVIRUS RECEPTOR GENE ENHANCE THE SUSCEPTIBILITY OF ADENOVIRUS INFECTION

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Alhamdullilah…

Segala puji-pujian kehadrat Allah s.w.t

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ABSTRACT

Gene-based therapies promise the potential to target the explicit gene delivery and expression to target cell populations. Adenoviral vectors are presently being tested clinically as a new strategy for the treatment of cancer. However, an important determining factor for the successful entry of such adenoviruses into target cells is expression of the Coxsackie virus and Adenovirus Receptor (CAR) at the cell surface. This raises the possibility that those gene-based therapy face the greatest therapeutic challenge might be the least susceptible to infection with therapeutic adenoviruses. If effective strategies can be implemented to boost CAR expression and hence the presence of primary receptor at the cell surface, this could prove most useful to adenovirus-based gene transfer. Therefore, this study aimed to possibly boost the expression of CAR specifically on CAR-negative Chinese Hamster Ovary (CHO) cell lines and evaluate their biological function by examining their susceptibility to transduction and infection of Adenovirus type 5. Initially, CHO cell line were transfected with human CAR cDNA fragment tagged with red fluorescent protein (DsRed) and sequentially selected with G418 antibiotic to generate stable cell line containing CAR-DsRed. Several transfection reagents such as GeneJuice (Novagen), Lipofectamine^{TM} 2000 (Invitrogen) and Xtreme HP plasmid DNA (Roche) were used for transfection study using flow cytometry. In addition, the expression of CAR on CHO-CAR-DsRed cell was determined by inverted fluorescent microscope, qRT-PCR and western blotting and antibody-blocking assay to verify the role of CAR in CHO-CAR-DsRed cells. CHO-CAR-DsRed cells were further examined by infecting wild-type adenovirus type 5 (wt-Ad5) and transduction recombinant adenovirus type 5 tagged with enhanced green fluorescent protein (Ad5EGFP) and their susceptibility were observed by using cytopathic effect and Giemsa staining. Flow cytometry analysis, showed that transfection efficiency of Xtreme HP (39.25±1.00 MFI) was higher than Lipofectamine^{TM} 2000 (17.1±1.00 MFI) and GeneJuice (11.1±1.00 MFI). The stable CHO-CAR-DsRed cells were estimated at average of 68.9%±1.12 MFI by transduction of Ad5EGFP and supported by evidence of infectibility of wt-Ad5 from Giemsa staining. Moreover, blocking of CAR expression on CHO-CAR-DsRed showed negative susceptibility to Ad5EGFP. These findings suggested that the strategy could be implemented to augment CAR expression and enhance the presence of primary cell surface receptor, as this could ornament the cell's susceptibility to adenovirus infection and beneficial for adenovirus-based gene therapy.

ABSTRAK

Terapi berasaskan gen menjanjikan potensi untuk sasaran penyampaian gen yang tepat dan ekspresi kepada sasaran populasi sel. Vektor Adenoviral kini sedang diuji secara klinikal sebagai strategi baru untuk rawatan kanser. Walaubagaimanapun, penentuan kejayaan Adenovirus masuk ke dalam sel sasaran adalah ekspresi "Coxsackie virus and Adenovirus Receptor" (CAR) di permukaan sel. Ini meningkatkan kemungkinan bahawasanya terapi berasaskan gen terapeutik menghadapi cabaran terbesar boleh jadi kurangnya penerimaan terhadap jangkitan adenoviruses terapeutik. Jika strategi yang berkesan boleh dilaksanakan bagi peningkatan ekspresi CAR dan dengan kehadiran reseptor utama di permukaan sel, sekaligus memberi pembuktian yang sangat berguna bagi terapi gen berasaskan Adenovirus. Oleh itu, matlamat kajian ini bagi berkemungkinan meningkatkan ekspresi CAR pada negatif CAR iaitu sel Ovari Hamster Cina (CHO) dan memeriksa kecenderungan mereka kepada transduksi dan jangkitan Adenovirus jenis 5. Pada mulanya, sel CHO transfeksi dengan jujukan CAR manusia cDNA dilabelkan dengan protein merah pendarfluor (DsRed) dan pemilihan berterusan dengan G418 antibiotik untuk menjana barisan sel stabil yang mengandungi CAR-DsRed. Beberapa reagen transfeksi seperti GeneJuice (Novagen), Lipofectamine^{TM} 2000 (Invitrogen) dan Xtreme HP plasmid DNA (Roche) reagen transfeksi digunakan bagi kajian sitometri aliran. Di samping itu, ekspresi CAR pada sel CHO-CAR-DsRed boleh ditentukan oleh mikroskop pendarfluor songsang, qRT-PCR, pewarnaan western dan esei penyekatan-antibodi untuk mengesahkan peranan CAR dalam sel CHO-CAR-DsRed. CHO-CAR-DsRed seterusnya dikaji dengan menjangkiti adenovirus kumpulan 5 jenis-liar (wt-Ad5) dan trasnduksi rekombinan Ad5 berlabel dengan peningkatan fluoresen protein berwarna hijau (Ad5EGFP) dan penerimaan jangkitan telah diperhatikan telah menggunakan kesan *cytopathic* (CPE) dan perwarnaan oleh Giemsa. Analisis sitometri aliran, memperlihatkan bahawa kebolehan transfeksi Xtreme HP (39.25 ± 1.00 MFI) adalah tertinggi berbanding kepada Lipofectamine^{TM} 2000 (17.1 ± 1.00 MFI) dan GeneJuice (11.1 ± 1.00MFI). Sel-sel CHO-CAR-DsRed yang stabil telah dijangkakan pada purata 68.9% ± 1.12 MFI oleh transduksi daripada Ad5EGFP dan disokong dengan pembuktian oleh jangkitan daripada jenis liar-Ad5 melalui pewarnaan Giemsa. Selain itu, penyekatan CAR menunjukkan pemerhatian yang negatif terhadap kecenderungan mana-mana Ad5EGFP. Kepentingan penemuan ini dapat memberi kesimpulan bahawa strategi ini boleh dilaksanakan untuk menghadirkan ekspresi CAR dan meningkatkan kehadiran reseptor primer di permukaan sel, justeru memudahkan penerimaan jangkitan adenovirus terhadap sel dan sekaligus bermanfaat untuk terapi gen berasaskan Adenovirus.